to work, it might be considerably more honest and more civilized than the unresponsible and often heartless de facto rationing of care that is now occurring, seemingly for lack of a better alternative.

MSMW

The Breadth of In Vitro Fertilization and Embryo Transfer

IN VITRO FERTILIZATION is clearly an idea whose time has come. It has thus far had profound ramifications on all aspects of reproductive medicine. It has influenced patient management and evaluation and shed new light on underlying pathophysiologic mechanisms that were previously a mystery. Consider the patient with unexplained infertility. As part of her workup and management, she may now have a cycle of hyperstimulation of oocytes based on the principle learned from in vitro fertilization that the more embryos placed in the uterus, the more likely a conception is to occur in any individual cycle. If all other treatment modalities fail, this patient may then go into an in vitro fertilization program which now presents a new treatment for her, with the potential of obviating the dead end met by previous procedures. This procedure has changed the face of the practice of reproductive endocrinology in a way that nothing else has.

The symposium "Extracorporeal Fertilization and Embryo Transfer in the Treatment of Infertility," appearing elsewhere in this issue, looks at but one aspect of this controversial area in that it compares and contrasts embryo transfer after in vivo fertilization versus embryo transfer after in vitro fertilization. Before embarking on a discussion as to the contrast between these two clinical processes, one is confronted with the great similarity seen. Eventually, fertilization and embryogenesis are the same in both procedures. It is only incubation and embryo transfer that differ in a technical way and the genetic makeup of the fetus that differs in a substantive way.

Critical to both processes is fertilization: once an oocyte is penetrated by a sperm, the second meiotic division occurs. Cortical granules are then activated which prevent penetration of further sperm into the ooplasm. This, as Zamboni describes, is the cortical reaction. Time is required after ovulation occurs for the oocyte to become ready to receive the spermatozoon. This usually occurs in the ampullary portion of the tube. This one biological condition provides both strict constraints on the timing of ovum capture for extracorporeal fertilization and also a window by which delayed exposure to sperm can be determined. After the oocytes are collected, they are inspected in the laboratory; if they are mature or intermediate based on cumulus dispersion, then exposure to spermatozoa within six hours is the rule. If they are immature, the eggs are allowed to incubate for 24 hours and are then exposed to spermatozoa. Trounson and co-workers in 1982 reported on the effectiveness of delayed insemination in an in vitro fertilization procedure.[1] This description allows for the less precise timing of ovum capture. By allowing immature eggs to mature in vitro for 24 hours, these, too, can be inseminated and result in embryos for transfer and eventual pregnancy. In some groups the number of immature eggs captured is as high as 20%.

Grading eggs as to maturity based on cumulus dispersion, simply stated, is that the more mature the egg, the more likely is the cumulus to be dispersed, thus facilitating sperm penetration. Studies have shown that in stimulated cycles, a discrepancy may occur between oocyte maturity as judged by cumulus dispersion and actual oocyte maturity based on ovum architecture.[2] Acrosomal enzymes help disperse the cumulus mass, thus aiding fertilization. Once the sperm head is in contact with the zona pellucida, the acrosome is released as protease, destroying the "last barrier separating (sperm) from the oocyte." The two pronuclei then undergo syngamy. Genetic material between the two gametes is mixed, followed by cleavage of the cell and eventual embryogenesis.

In the most successful centers around the world that provide in vitro fertilization and embryo transfer, the success rate, defined as a clinical pregnancy occurring after a laparoscopy to retrieve ovum, is approximately 20%. The viable pregnancy rate approaches 17%.[3] Therefore, the thrust in this procedure must be towards improving success rates per cycle. How can we best accomplish this, and where should this flurry of activity surrounding in vitro fertilization and embryo transfer be aimed? Basically, there are two important time frames to be investigated: one is the period of ovulation induction and the second is implantation. Little to nothing is known about the implantation of the human embryo, for obvious reasons. Given our current level of information, it would be hard to even construct sophisticated clinical studies to look at implantation of the human embryo. For this reason, scrutiny of ovulation induction as a reflection of normal embryogenesis takes on significance. In contrast, the laboratory environment for embryogenesis has not in the past few years been accorded the importance that it should.

Ovulation and ovulation induction are a complicated cascade of events, and attempts are being made to determine markers of successful ovulation induction. These attempts include monitoring peripheral estrogen levels, doing ultrasonography and studying follicular fluid contents. Through the use of cell culture, the performance by various cellular components of the follicle, including the granulosa cells and the cumulus corona complex, is being investigated. Peripheral blood measurements have shown that estrogen levels, which rise with the administration of human chorionic gonadotropin, correlate with increased success rates.[4] Cyclic adenosine monophosphate has been evaluated in follicular fluid and seems to correlate inversely with maturity of the oocyte and thus pregnancy rates.[5] Studies that involve the cumulus corona complex show that a high ratio of estradiol to testosterone correlates with higher fertilization and cleavage rates.[6]

Ovulation induction, therefore, remains the key, at least today, for successful in vitro fertilization. As best we can tell, proper induction of ovulation with a normal surrounding hormonal milieu best correlates with normal embryo development and pregnancy. What can be done to manipulate normal ovulation induction and make it successful, and what goals are we trying to achieve? The ideal would be four oocytes, all at the same level of maturity (preovulatory), surrounded by a system with a hormonal milieu conducive to implantation. The problem is that with most ovulation induction methods used, various cohorts of follicles are recruited as a function of time, making asynchrony a significant problem. Attempts have been made to change the treatment regimen in various ways but none have been satisfactory. A theoretical concept in humans to control ovulation more precisely would be to turn

off the endogenous stimulus to the ovary entirely, using a gonadotropin-releasing hormone antagonist, and then to superimpose on this ovary ovulation induced by the use of exogenous gonadotropins.

In 1985 Mohr and associates[7] reported deep freezing and thawing of human embryos with transfer; 68 embryos were transferred into 48 patients, resulting in 9 pregnancies. This breakthrough certainly will have wide ramifications. If success continues, it has two distinct advantages: only one capture procedure is necessary to do embryo transfers in multiple cycles, and transfers can be done in unstimulated cycles. It is possible that the high levels of estrogen created by whatever ovulation induction mechanism is used creates a deleterious effect on the endometrium. A major pragmatic goal in the field of in vitro fertilization and embryo transfer, in addition to improved ovulation induction, is to be able to predict the outcome, selecting only those patients who will be successful in achieving a pregnancy through this procedure.

Clinical factors are also extremely important in predicting outcome and, for referring physicians, these are of paramount importance. Which patients are most likely to get pregnant with this system? The four most common reasons for infertility for which patients are referred for in vitro fertilization and embryo transfer are tubal factor, male factor, idiopathic infertility and endometriosis. As Meldrum has described in this issue, pregnancy rates for those patients who have idiopathic infertility are in some systems surprisingly lower than would be expected. This, of course, is an aspect of this procedure that is filled with excitement and expectation. The possibility clearly exists that new causes of infertility will emerge—that is, failure of fertilization opens up whole new areas of manipulating basic science information to facilitate this emergence. The injection of sperm heads under micromanipulation, although quite difficult, will certainly be a possibility for certain infertile couples in the future.

Other clinical factors such as age also are important and questions surrounding the influence of aging and decreasing fertility certainly will be answered in part by new information obtained from these procedures.

To fully understand the effects of in vitro fertilization and embryo transfer procedures on clinical reproductive science, one has only to look at male-factor infertility. In the past, many treatments were tried, with little success, but today techniques that enhance sperm fertilization efficiency are used and have permitted improvement in an area previously bogged down by lack of success. In fact, the methods used to improve sperm quality for in vitro fertilization and embryo transfer have been applied to the oligospermic male group, and intrauterine insemination has been associated with measured success.[8]

Buster's experience in a "nonsurgical ovum transfer program" certainly seems to have some distinct advantage, the primary one being that a laboratory is not needed to facilitate fertilization, cleavage and embryogenesis. A proper start in considering this procedure would be a more precise term because it is really not transfer of an ovum; it is nonsurgical transfer of an embryo. This has been quite successful in Buster's hands but has one major disadvantage, that of adding a new gene pool into a family desirous of having a child. It does have specific indications, the best being a woman who has no ovaries and would very much like to carry a child, especially one who was the result of an egg fertilized by her husband. Unfortunately, little so far has been learned in regards to understanding reproduction from the "nonsurgical ovum transfer program." Technically, it can be done—that has been shown—but what we have learned about basic reproductive biology is less complete when compared with what we have learned in a brief period of time about "classic" in vitro fertilization.

Obviously, lessons in donor fecundity comparing various male donors will emerge, finally defining fertility not as an absolute but as a relative biologic characteristic. A calculating question that intrigues us all is whether there are superfertile men and superfertile women and subfertile men and women, and if a subfertile man marries a superfertile woman, will the results be normal fertility for the couple? Buster has the tools to answer this important question.

Most of this new technology has raised a tremendous number of moral, ethical and legal questions. The history of new issues seems to be that once a concept has been presented, society works out the problems in various ways, and we move along to new problems. Our own experience has been one of tremendous resistance in getting a program of in vitro fertilization and embryo transfer established based on ethical compunctions on the part of the community. After three years, it is now a well-accepted procedure, with few questioning its validity or ethical implications. On the other hand, we have recently embarked on an embryo-freezing program that has stirred up the same feelings and legitimate questions as did our past foray into the general community with a new idea.

The future for in vitro fertilization and embryo transfer is vast. It is a field that is growing more rapidly than one can keep abreast of. It tantalizes the imagination to think about what reproductive biologists and genetic engineers may be able to accomplish. As an ethicist, I find the problems are equally stimulating. This is a good thing for humankind and I hope that it will not be exploited. It can provide help for the incurably infertile and perhaps cures for some as-yet-incurable genetic diseases. The spinoffs in information about reproduction will revolutionize previously held dogma and eventually eliminate infertility as one of our major sources of grief.

ALAN H. DeCHERNEY, MD
Professor of Obstetrics and Gynecology
Director, Division of
Reproductive Endocrinology
Yale University School of Medicine
New Haven, Connecticut

REFERENCES

1. Trounson AO, Mohr LR, Wood C, et al: Effect of delayed insemination on in vitro fertilization, culture and transfer of human embryos. J Reprod Fertil 1982; 64:285-294
2. Laufer N, DeCherney AH, Haseltine FP, et al: Steroid secretion by the human egg-corona-cumulus complex in culture. J Clin Endocrinol Metab 1984; 58:1153-1157
3. Seppala M: The World Collaborative Report on In Vitro Fertilization and Embryo Replacement: Current state of the art in January 1984, *In* In Vitro Fertilization and Embryo Transfer. Ann NY Acad Sci 1985; 442:558-563
4. Laufer N, DeCherney AH, Tarlatzis B, et al: Association between preovulatory serum 17β-estradiol pattern and conception in human menopausal gonadotropin-human chorionic gonadotropin. Fertil Steril, in press
5. Tarlatzis BC, Laufer N, DeCherney AH, et al: Adenosine 3′,5′-monophosphate levels in human follicular fluid: Relationship to oocyte maturation and achievement of pregnancy after in vitro fertilization. J Clin Endocrinol Metab 1985; 60:1111-1115
6. Laufer N, Tarlatzis B, DeCherney AH: Steroid secretion by human oocyte-corona-cumulus complexes associated with conceptions following in vitro fertilization. J Vitro Fertil Embryo Transfer 1985; 2:156-161
7. Mohr LR, Trounson A, Freemann L: Deep-freezing and transfer of human embryos. J Vitro Fertil Embryo Transfer 1985; 2:1-10
8. Huszar G, Makler A, Corrales M, et al: Intrauterine insemination: Experience after 1,000 cycles. Proceedings of the 32nd Annual Meeting of the Society for Gynecologic Investigation, Phoenix, March 1985, p 182